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REMARKS

Claims 1-8 are pending. Claims 5-7 have been amended to correct minor errors. Thus, claims 1-8 will be pending and under examination upon entry of this Amendment.

In view of the arguments set forth below, applicants maintain that the Examiner's rejections made in the February 27, 2004 Advisory Action have been overcome, and respectfully request that the Examiner reconsider and withdraw same.

Rejections Under 35 U.S.C. §103(a)

The Examiner rejected claims 1-5, 7, and 8 under 35 U.S.C. §103(a) as allegedly unpatentable over Shuman (1992) in view of Bjornson et al. (1994), of record.

In response to the Examiner's rejection, applicants respectfully traverse. Applicants maintain that the cited references fail to support a *prima facie* case of obviousness for the reasons of record and for the additional reasons set forth below.

Specifically, applicants maintain that the Examiner has failed to establish a *prima facie* case of obviousness because the method of Shuman, combined with the teachings of Bjornson, does not create a reasonable expectation of success for practicing the claimed methods. Applicants note that "[a] reasonable expectation of success is the standard by which obviousness is determined." M.P.E.P. §2141.

The rejected claims provide methods for detecting the activity of an RNA helicase utilizing fluorescently labeled duplex RNA.

Shuman teaches a method for detecting RNA helicase activity utilizing *radiolabeled* duplex RNA.

Bjornson teaches a method for detecting DNA helicase activity utilizing fluorescently labeled duplex *DNA*.

Applicants understand the Examiner's rejection to be based on the assertion that one of skill in the art could "simply [use] the detection methodology of Bjornson et al. in the method of Shuman" to arrive at the claimed invention.

Applicants respectfully disagree with the Examiner's position for the following reasons. In order to substitute the detection methodology of Bjornson in the method of Shuman, one of skill would have to make fluorescence-labeled duplex RNA that could be detected in the method of Shuman. Importantly, Bjornson does not teach how to make fluorescence-labeled duplex RNA. However, even if art-recognized techniques for fluorescent labeling of DNA could be used to produce a fluorescence-labeled RNA comparable to the DNA of Bjornson, applicants maintain that the Examiner has not demonstrated a reasonable expectation of success in using the fluorescence-labeled RNA in place of the radiolabeled RNA in the method of Shuman.

Shuman relies on the detection of very small amounts of radiolabeled RNA. For example, Shuman uses femptomolar quantities of labeled duplex RNA in the helicase assays of Figures 3 and 4. The Examiner has not shown that such small amounts of fluorescence-labeled RNA would have been detectable in the method of Shuman, because the Examiner has not demonstrated that fluorescent labels are as sensitive as radiolabels. For example, radiolabels have a lower limit of detection in the femptomolar to picomolar (10^{-14} - 10^{-12}) range

(see Freifelder at page 129, attached hereto as **Exhibit A**). In contrast, according to the art, fluorescence detection is several orders of magnitude less sensitive. For example, the detection of the fluorescent molecule fluorescein *itself* at 2 x 10^{-6} M was considered "an example of extraordinary sensitivity" (see Freifelder at page 569 in Exhibit A). Thus, applicants maintain that one of skill would not have had a reasonable expectation of success in substituting fluorescence-labeled RNA for the radiolabeled RNA in the method of Shuman, even if it had been within the routine skill of the art to produce a suitable fluorescence-labeled RNA, which applicants do not concede.

Applicants maintain that the art at the time of filing did not provide a reasonable expectation of success in producing a fluorescence-labeled RNA suitable for use in a helicase assay. Importantly, it is necessary to incorporate enough fluorescent label into the RNA to detect very small amounts of labeled RNA in the assay. Specifically, the assay requires the detection of labeled RNA in the nanomolar (10^{-9} M) or subnanomolar range. This is because at higher concentrations, reannealing will occur at a significant rate during the assay, a fact that was known in the art of helicases (see Bjornson at page 14314 and the specification at page 38, lines 27-28).

Thus, to produce a labeled RNA suitable for fluorescence detection in the nanomolar range, a high efficiency labeling reaction was required. Applicants maintain that the success of Bjornson in producing a fluorescently labeled DNA suitable for use in a helicase assay does not provide a reasonable expectation of success in accomplishing the same with RNA. In this regard, the Examiner has not shown how the chemical differences between RNA and DNA would permit the direct

application of DNA labeling techniques to RNA. For example, the 3' terminus of RNA is chemically different from that of DNA, and the Examiner has not shown how this would not require the use of different labeling methods than those used for DNA. Applicants note that both the 3' and 5' ends of the duplex DNA are labeled in Bjornson. With respect to the 5' terminus, the Examiner has not shown that methods for labeling the 5' terminus of RNA with fluorophors were well-established at the time of the invention.

In summary, applicants maintain that the Examiner has failed to show that the state of the art in fluorescent labeling of RNA at the time of applicants' invention provided a reasonable expectation of success for producing a fluorescently labeled duplex RNA that could be used in the claimed methods. Accordingly, applicants maintain that prior to the instant invention, one of skill would not have been motivated to apply the method of Bjornson to RNA helicases, nor would one have been motivated to attempt the method of Shuman using fluorescence-labeled RNA, because there was no reasonable expectation that either combination would work.

The Examiner also rejected claim 6 under 35 U.S.C. §103(a) as allegedly unpatentable over Shuman (1992) in view of Bjornson et al. (1994) and further in view of Vargo et al.

In response, applicants respectfully traverse for the reasons of record and for the following additional reasons.

According to the Examiner, Vargo teaches the particular fluorescent labels recited in claim 6. This teaching of Vargo, when combined with Shuman and Bjornson, does nothing to overcome the lack of motivation or expectation of success discussed above. The lack of motivation to produce a

fluorescently labeled RNA, detectable at nanomolar concentrations, has been noted. Applicants maintain that Vargo, combined with Shuman and Bjornson, fails to create a reasonable expectation of success in view of these obstacles.

In sum, applicants maintain that the cited references combined fail to create either a motive to combine their respective teachings or a reasonable expectation of success. Accordingly, applicants maintain that the claimed methods are not *prima facie* obvious over Shuman (1992) in view of Bjornson et al. (1994) and Vargo.

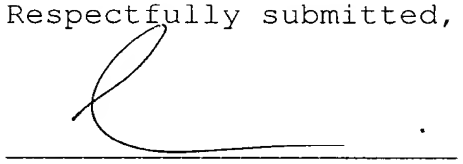
Summary

In view of the remarks made herein, applicants maintain that the claims pending in this application are in condition for allowance. Accordingly, allowance is respectfully requested.

No fee, other than the enclosed $595.00 fee, is deemed necessary in connection with the filing of this Amendment. However, if any additional fee is required, authorization is hereby given to charge the amount of such fee to Deposit Account No. 03-3125.

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicants' undersigned attorneys invite the Examiner to telephone them at the number provided below.

Respectfully submitted,



John P. White
Registration No. 28,678
Alan J. Morrison
Registration No. 37,399
Attorneys for Applicants
Cooper & Dunham, LLP
1185 Avenue of the Americas
New York, New York 10036
(212) 278-0400

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5

Radioactive Labeling and Counting

Many biochemical analyses require the detection of minute (10^{-14}–10^{-6} mole) quantities of material. However, chemical tests are rarely responsive to less than 10^{-7} mole. This limitation has been alleviated by the development of radiotracer technology through which extraordinarily sensitive detection of radioisotopically labeled material has allowed studies of many substances in quantities of 10^{-12} mole to become routine. In addition, the use of radioactivity has permitted the development of powerful experimental approaches to various types of problems. Such approaches employ the *double-labeling* technique for following two substances simultaneously or for distinguishing two identical substances synthesized at different times; the *pulse-chase* method for following a substance at a time after its synthesis without the interference of material concurrently synthesized; and *exchange analysis* for measuring participation in reactions.

In this chapter, these and other techniques will be described in some detail. The methods for detecting and measuring radioisotopes will also be presented herein. Autoradiography has been excluded from this chapter because its technology and applications are rather different from the subject of this chapter. It is presented in Chapter 6.

Types of Radiation Used in Biochemistry

Nuclear radiation is a result of the spontaneous disintegration of atomic nuclei. Of the several kinds of emitted radiation those of importance in isotopic labeling are β particles (emitted electrons) and γ rays (photons).*

* Alpha particles are rarely used for two reasons: (1) they are difficult to detect because they are strongly absorbed by the samples themselves; and (2) there are few α-emitting isotopic labels that can be satisfactorily used for biological materials.

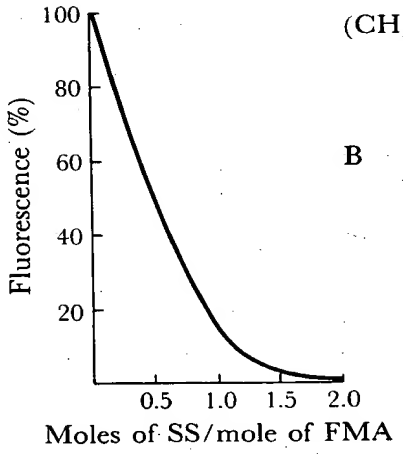
is incubated with cells containing the antigen and then washed away, examination of the cells by fluorescence microscopy will show fluorescence only where the antigen is present. This method is widely used to detect tumor antigens and to identify intracellular viruses. It is described more fully in Chapter 2 and an example is given in Figure 2-20.

Example 15-BB ☐ Assay of S—S bonds and SH groups.

In 1 M NaOH, the fluorescence of fluorescein mercuric acetate is quenched by disulfide bonds in protein. This is a relatively accurate means to assay these bonds. A protein with a known number of S—S bonds and SH groups (e.g., ribonuclease) must be used for calibration (Figure 15-15). At neutral pH, the fluorescence is quenched instead by SH groups but not S—S bonds.

Example 15-CC ☐ Enzyme assays.

Fluorescence assays, in which nonfluorescent substances are converted into fluorescent ones, or vice versa, exist for numerous hydrolytic enzymes (e.g., cholinesterase, lipase, hyaluronidase, and β-galactosidase), oxidative enzymes (e.g., aryl hydroxylases, peroxidases, and



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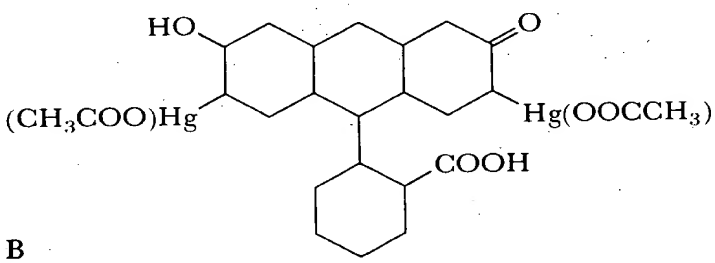

B

S-S/molecule

Protein	FMA titration	Chemical method
Human serum albumin	18	17
Lysozyme	3.9	4
Trypsin	5.9	6
Insulin	3.0	3
Chymotrypsinogen	5.3	5

C

Figure 15-15

A. Measurement of S—S bonds by the quenching of fluorescence of fluorescein mercuric acetate (FMA). B. The structure of the fluor. C. A comparison of the results with other methods. [From F. Karush, N. R. Klinman, and R. Marks, *Anal. Biochem.* 9(1964): 100–114.]

oxidases), transaminases, dehydrogenases, isomerases, kinases, and decarboxylases. An example of the extraordinary sensitivity of such assays can be seen in the work of Boris Rotman [*Proc. Natl. Acad. Sci. U.S.A.* 47(1961):1981–1991], who measured the activity of a *single* molecule of β-galactosidase. Purified β-galactosidase was diluted and dispersed into droplets of roughly 10^{-9} ml—each containing a known concentration of the nonfluorescent substance fluorescein di-(β-D-galactopyranoside). Hydrolysis of this substance yields fluorescein. The fluorescence of each droplet was measured with a microscope equipped with a photomultiplier and suitable apertures so that a single drop could be observed. In these droplets it was possible to detect fluorescein at 2×10^{-6} M, or 1.7×10^{6} molecules per droplet. Because one enzyme molecule hydrolyzes roughly 2×10^{5} molecules of substrate per hour, the fluorescein could be detected after approximately 10 hours. This method was then used to determine the number of enzyme molecules in a single *E. coli* bacterium.

☐ Quantitative measurement of DNA. **Example 15-DD**

The fluor ethidium bromide binds tightly to DNA; in so doing, the quantum yield increases substantially. This increase is linear throughout a wide range and the measurement of fluorescence intensity of an ethidium bromide solution containing small amounts of DNA can be used in a quantitative way to measure DNA concentration.

This enhancement of Q is also being used to detect DNA that has been electrophoresed in polyacrylamide and agarose gels (see Chapter 9, Figure 9-13). If a gel containing DNA is soaked in an ethidium bromide solution, it will take up the fluor. If the gel is then exposed to exciting light, the DNA bands become visible as regions of intense fluorescence.

☐ Detection of primary amines and peptides. **Example 15-EE**

In chromatography and electrophoresis, it is frequently necessary to detect primary amines such as amino acids and peptides. Several chemical techniques are available for this purpose but sometimes, when only a very small sample is available, these procedures are not adequate. When *o*-phthalaldehyde, a nonfluorescent substance, is mixed with primary amines, an intense blue fluorescence is produced. This is so sensitive that it has been possible to detect all of the spots of a protein fingerprint using only 10^{-5} g of protein.

SELECTED REFERENCES

Cantoni, G. C., and D. R. Davies, eds. 1971. *Procedures in Nucleic Acid Research*, vol. 2. Harper and Row. This contains several articles on nucleic acid fluorescence.

Chen, R. F., and H. Edelhoch. 1975. *Biochemical Fluorescence*, vol. 1 and 2. Dekker.